The effect of Kalanchoe pinnata on the cell migration of astrocytomas

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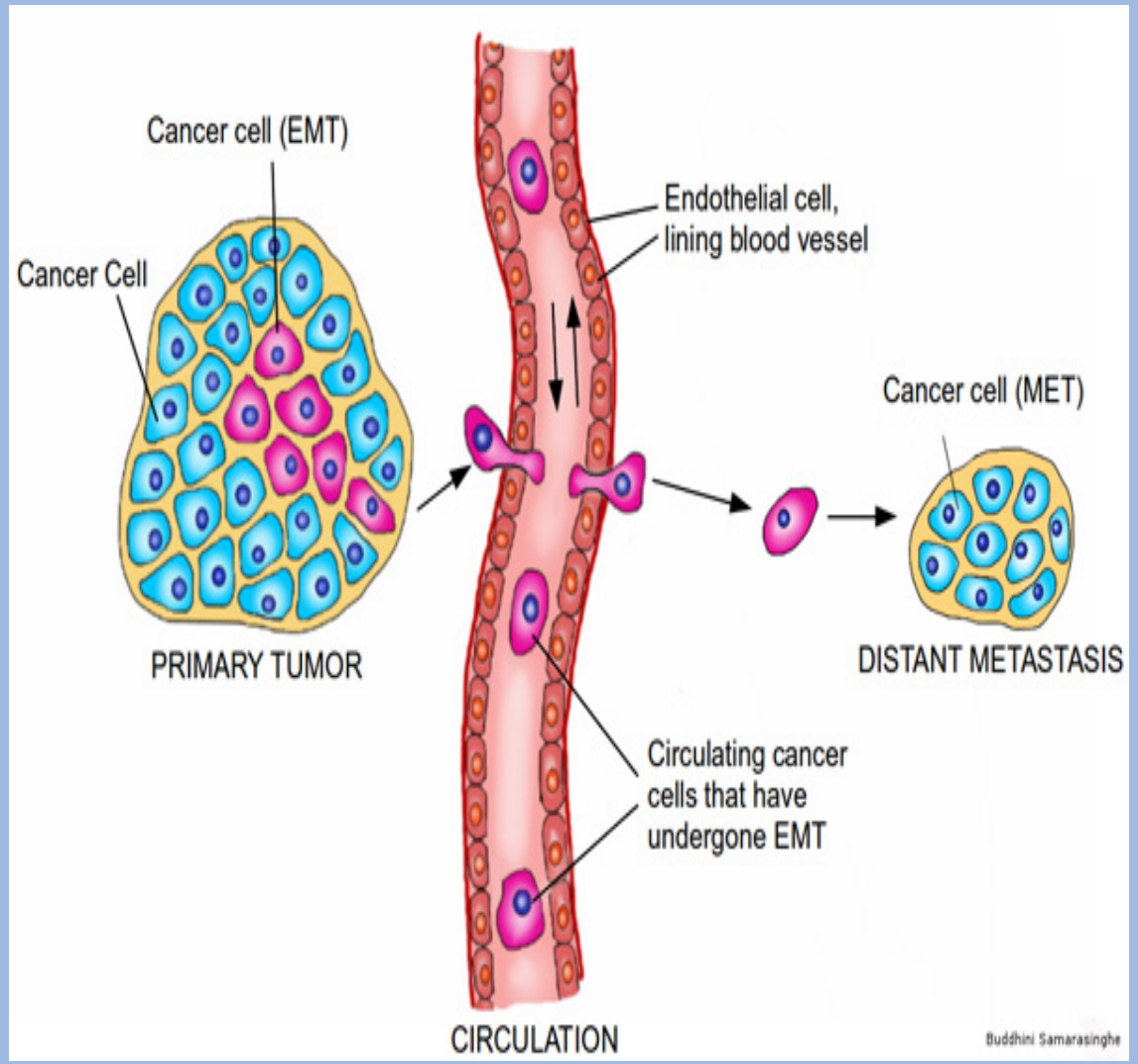
Research Problem

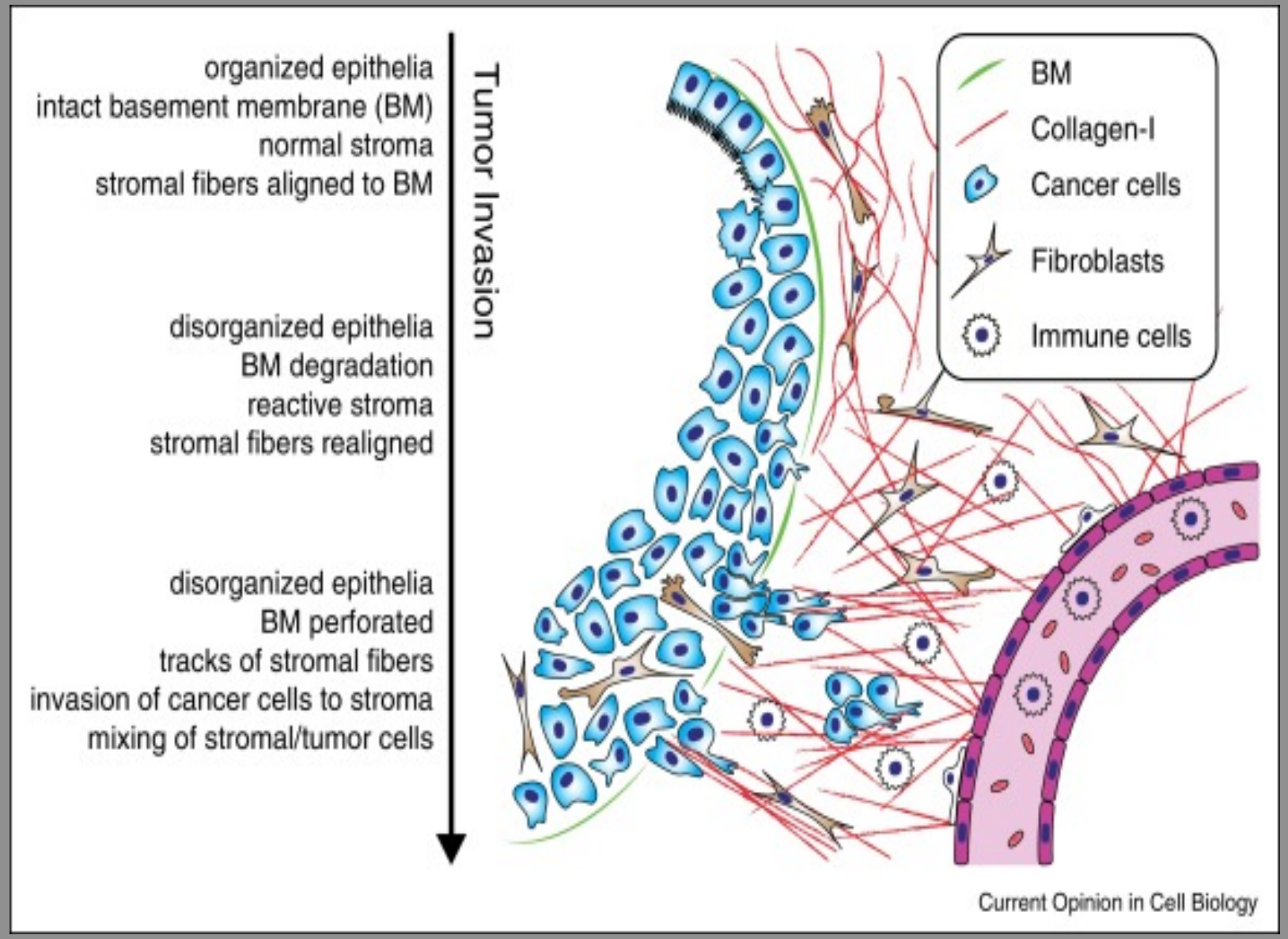
- Can treatment with an aqueous solution of Kalanchoe pinnata leaves inhibit cell migration on astrocytomas?

Applicability

- If cell migration is inhibited the metastatic process can be halted or prevented.



https://blogs.scientificamerican.com/guest-blog/the-hallmarks-of-cancer-6-tissue-invasion-and-metastasis/



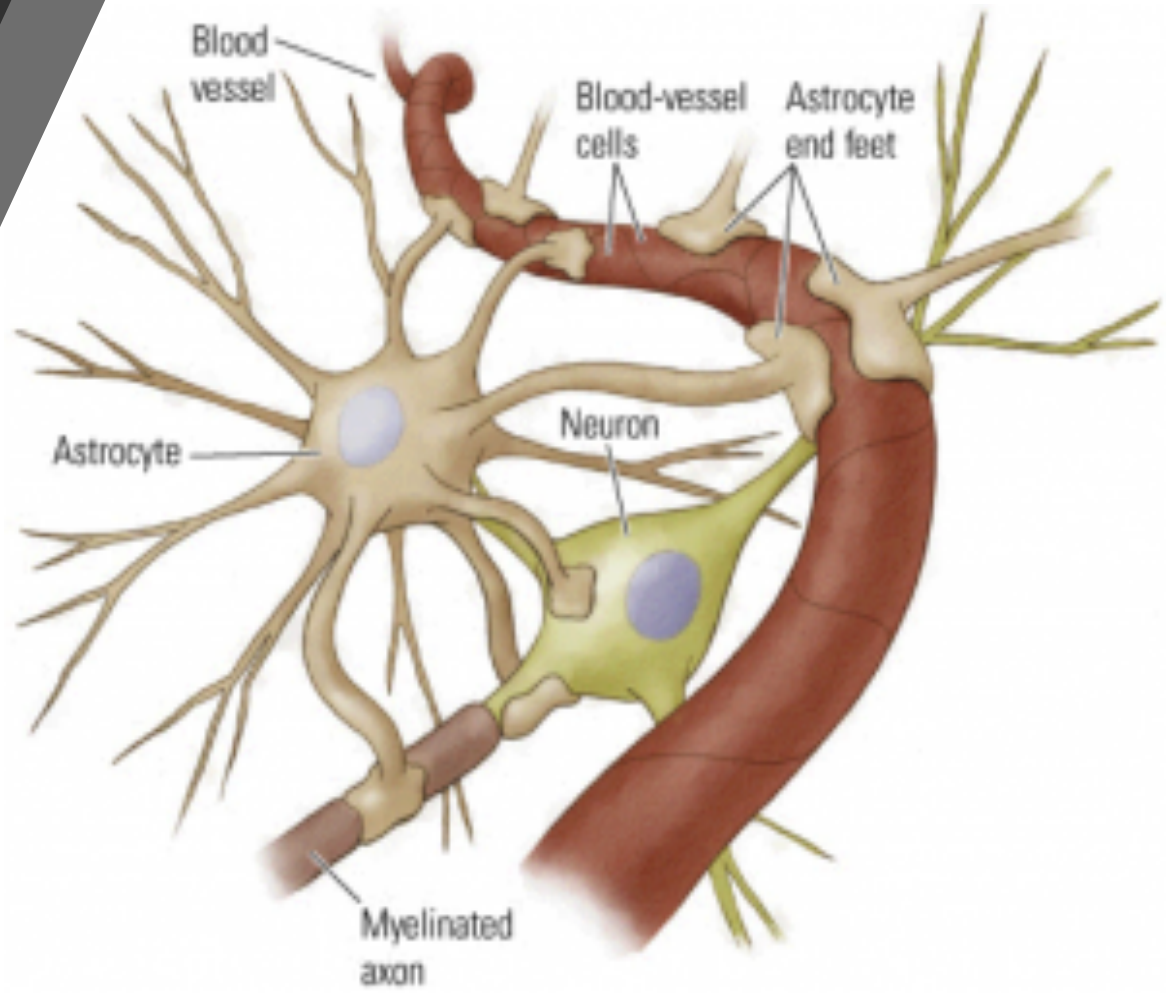
https://www.sciencedirect.com/science/article/pii/S0955067415000708

Background & Introduction

- Tumor invasion is the first stage of the metastatic process. During said phase cells begin to migrate away from the initial tumor and move towards blood and lymph vessels.

Background & Introduction

- Astrocytomas, from cell line 1321N1, are cancerous astrocytes.
  - Astrocytes are glial brain cells responsible for maintaining synaptic contact and the signaling capacities among neurons. They also represent a fundamental element in controlling blood flow and defining brain micro structure.



http://jonlieffmd.com/wp-content/uploads/2013/04/untitled211316472995722-300x243.png

Background & Introduction (continuation)

- Kalanchoe pinnata has show inhibitor characteristics against the Epstain-Barr virus mainly due to the cytotoxic activity that it's flavonoids and bufadienolides can carry out. Diagremontianin is a highly cytotoxic bufadienolide found in Kalanchoe. Other members of the Kalanchoe family, such as Kalanchoe tubiflora, have antiproliferative capabilities that make them viable for cancer treatments.

Methodology – Acquisition of leaf components

http://massspectrumbotanicals.net/wp-content/uploads/2013/04/KalPin.jpg

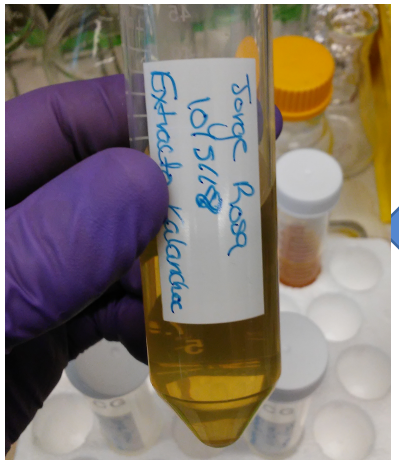
Lyophilization

Dilution

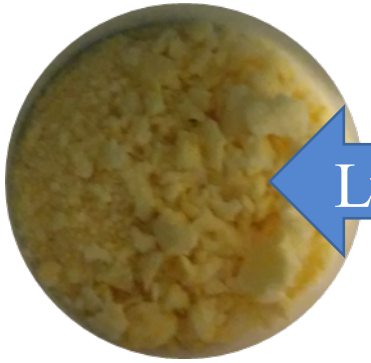
Filtration

Rotary evaporation

Centrifuge



Lyophilization

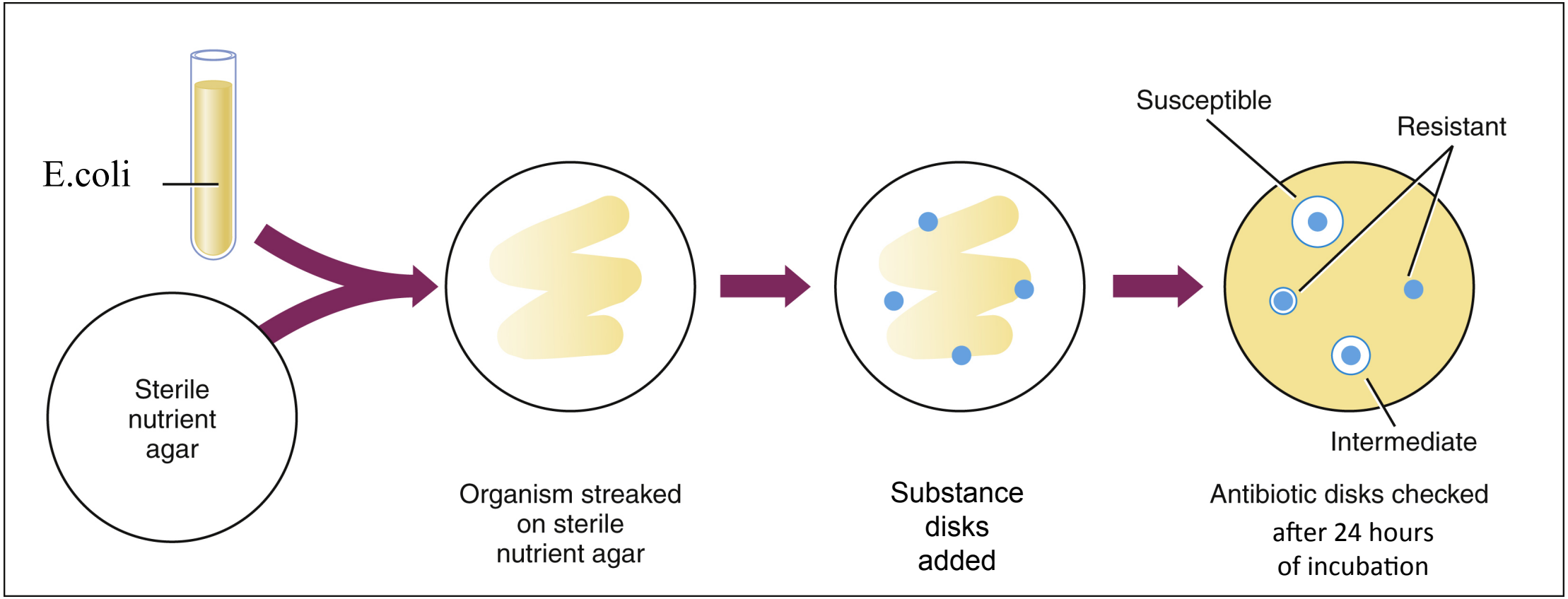


Methodology (continuation)

Antibiotic Susceptibility Test

- Kirby-Bauer Method
  - Organism: E. coli
  - Substances:
    - Gentamicin (Control 1) (upper left)
    - PBS (Control 2) (upper right)
      - Dilution (6µL/2mL)
    - KP-W (lower half)
      - 50% Serial Dilutions
        - 5% (0.25g/5mL)
        - 2.5% (0.125g/5mL)
        - 1.25% (0.0625g/5mL)
        - 0.625% (0.03125g/5mL)

Methodology – Antibiotic Susceptibility Test

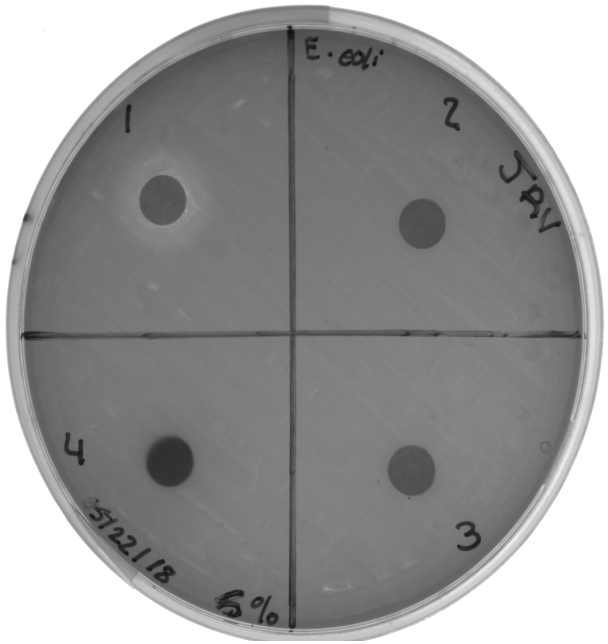


https://basicmedicalkey.com/wp-content/uploads/2017/01/B9781416066279000378_fx2.jpg

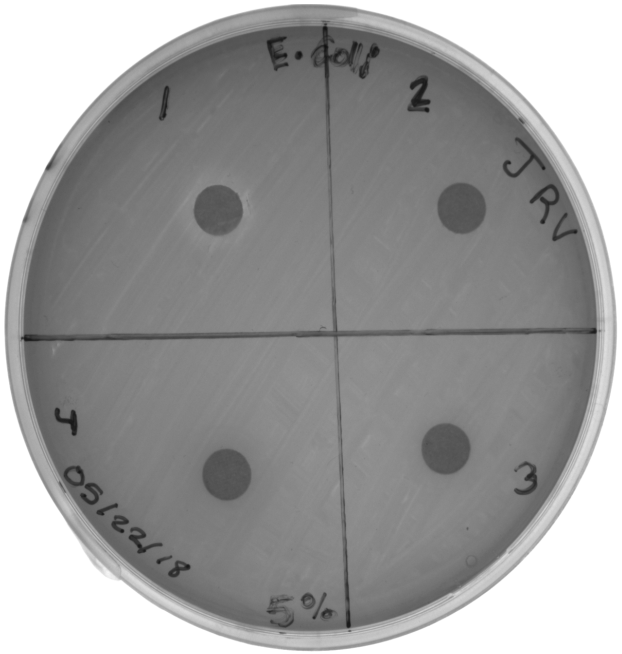
Variables

Independent- Substances (Gentamicin, PBS, KP-W),
Concentrations of KP-W
Dependent- Zone of inhibition (mm)
Constants- Organism; Observation period

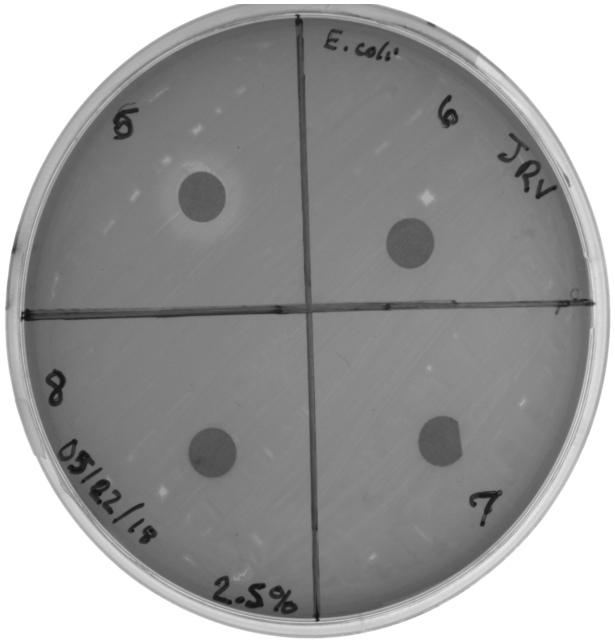
Antibiotic Susceptibility Test Results



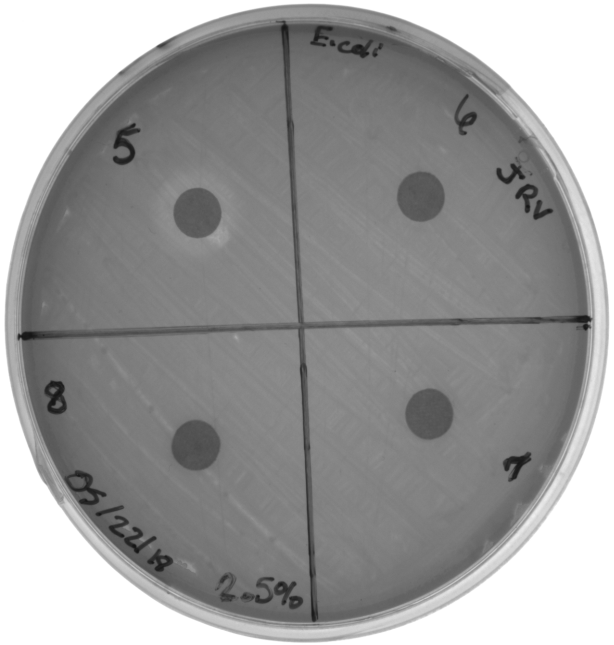
Kalanchoe pinnata 5%(1)



Kalanchoe pinnata 5%(2)

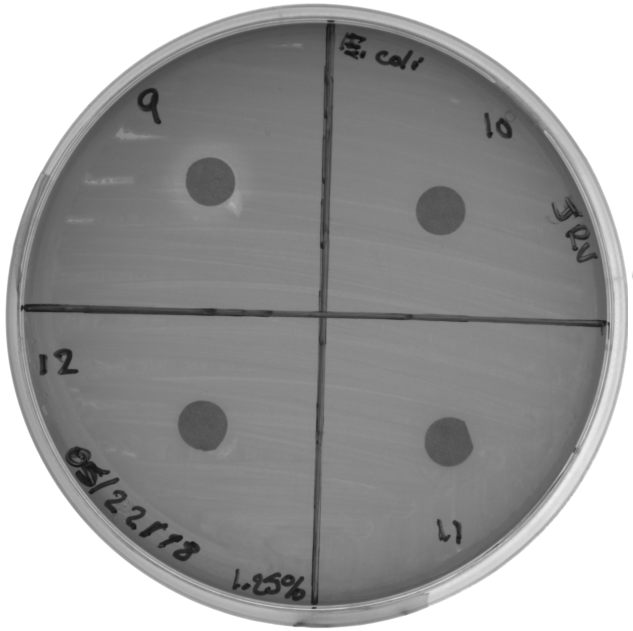


Kalanchoe pinnata 2.5%(1)

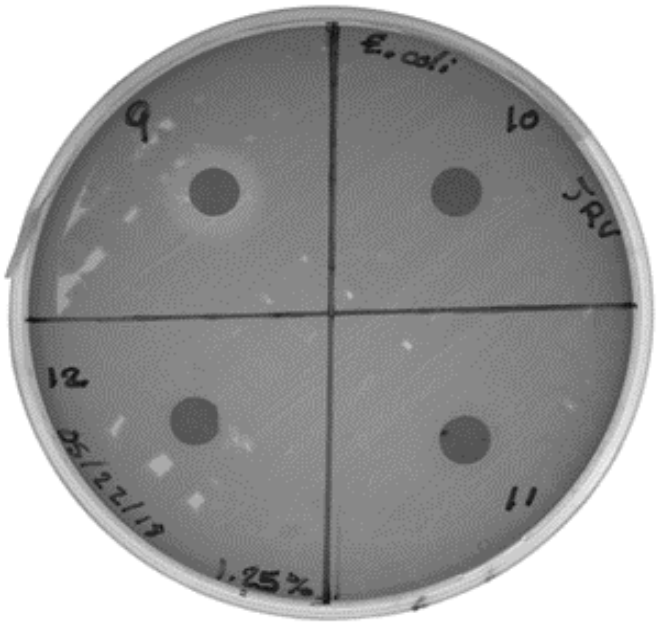


Kalanchoe pinnata 2.5%(2)

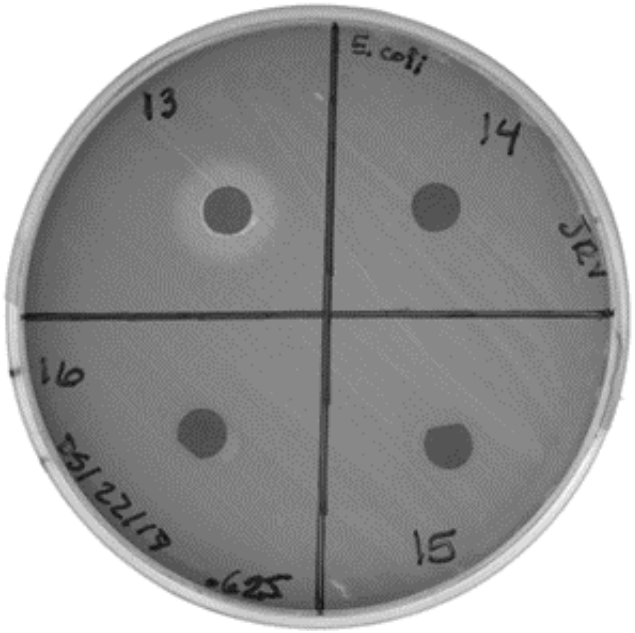
Antibiotic Susceptibility Test Results



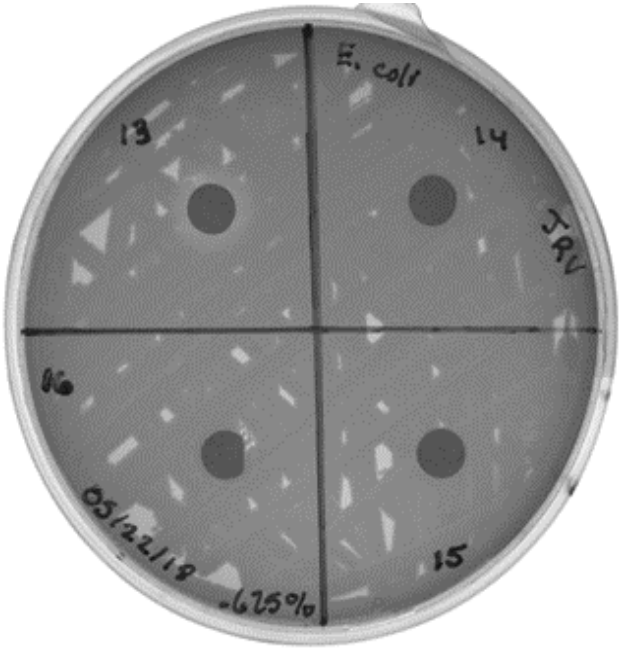
Kalanchoe pinnata 1.25%(1)



Kalanchoe pinnata 1.25%(2)



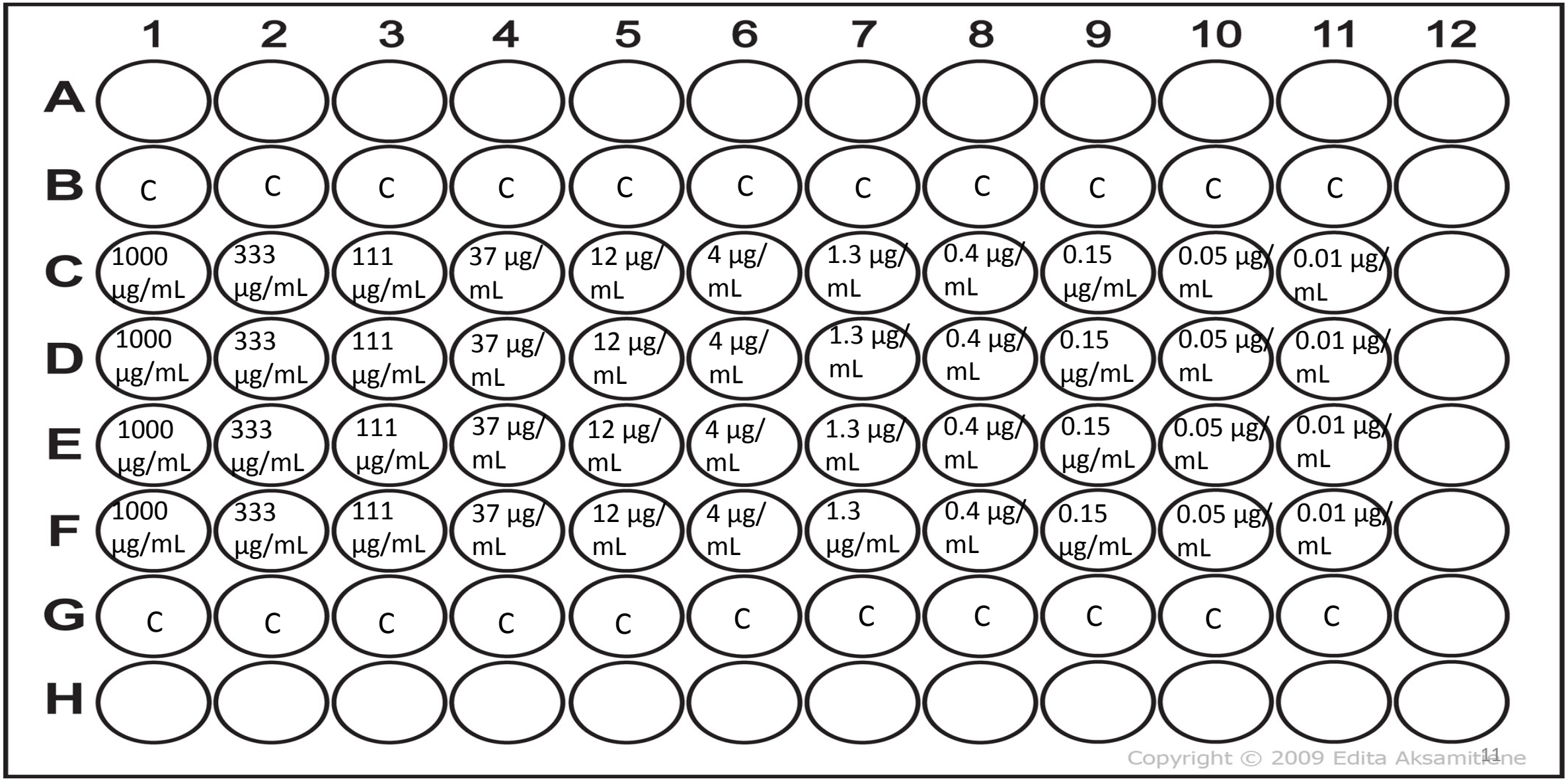
Kalanchoe pinnata 0.625%(1)



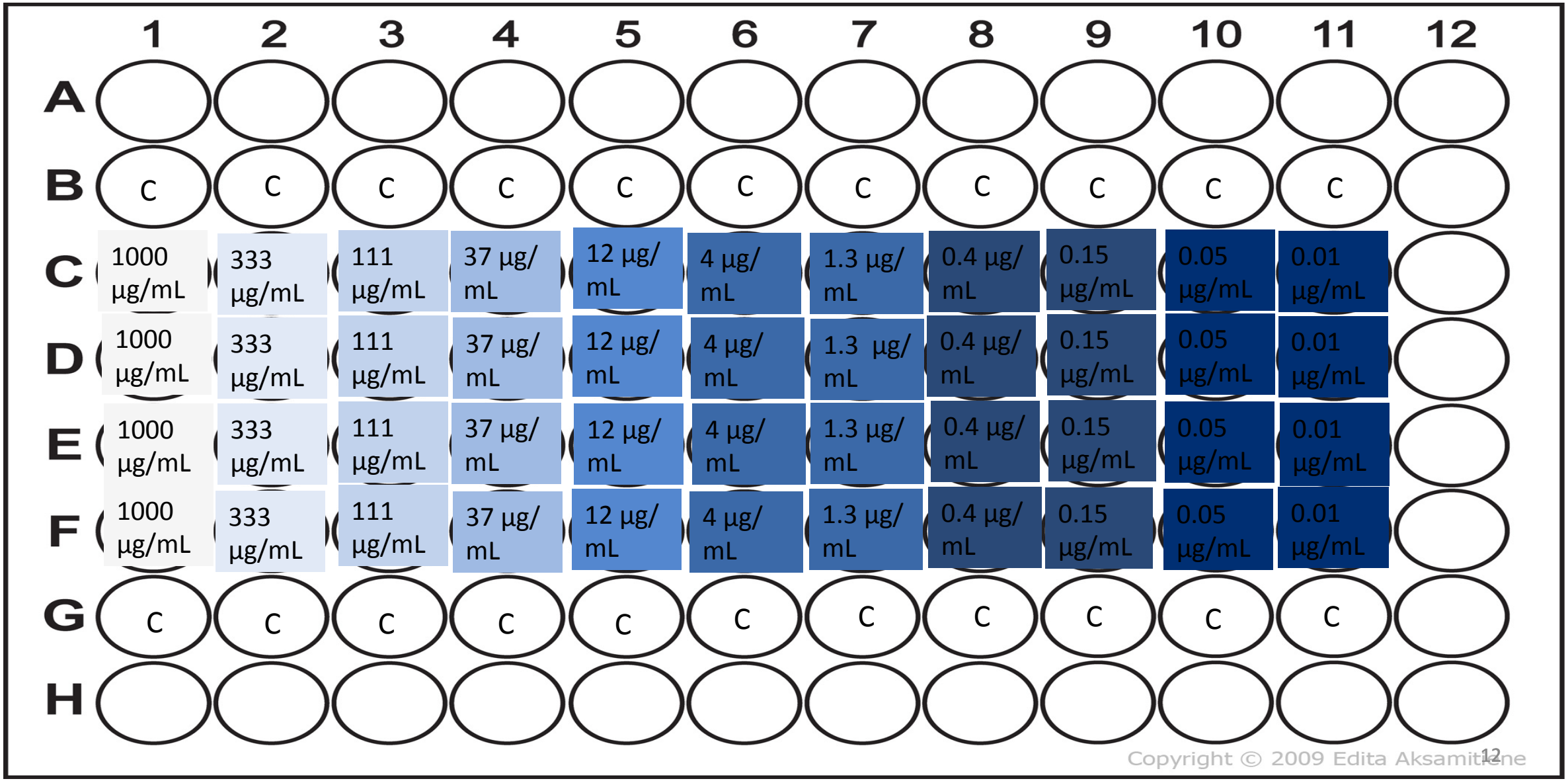
Kalanchoe pinnata 0.625%(2)

Conclusion: Due to the fact that antibacterial components in Kalanchoe pinnata are phenolic and highly hydrophobic it is likely that they were not present in the aqueous solution.

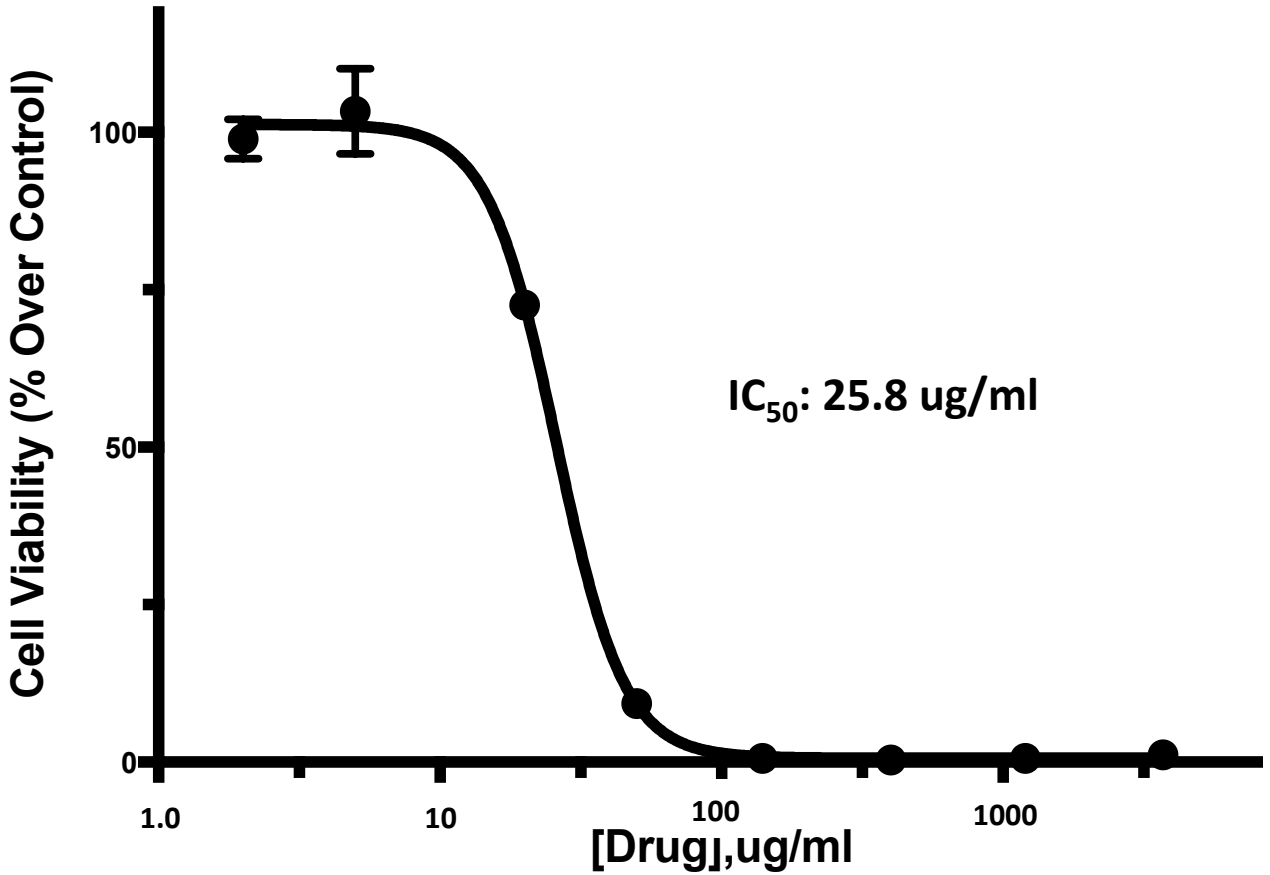
Methodology- Cytotoxicity Assay



Methodology – Cytotoxicity Assay



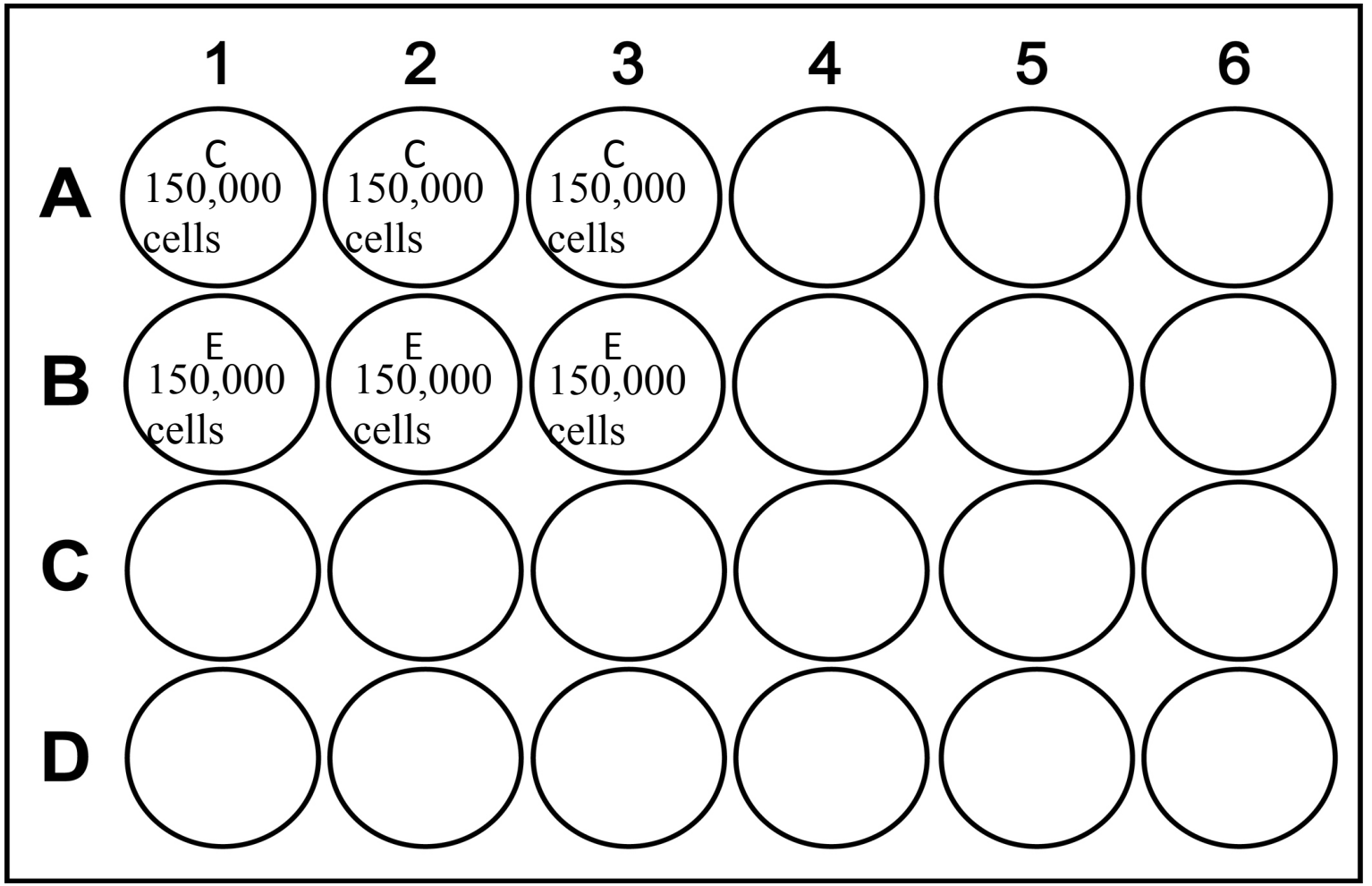
Cytotoxicity Assay Results and Analysis



- Variables
  - Independent – Concentrations of KP-W
  - Dependent – Percentage of cell viability
  - Constants – Cell line; Initial quantity of cells per well; Substance; Incubation period

Conclusion: IC50 show that a concentration of 25.8 µg/mL kills half of the total cells in the well . However, in order to test wound healing capabilities the concentration that will be used is 10 µg/mL since it does not eliminate any cells.

Methodology – Wound Healing Assay



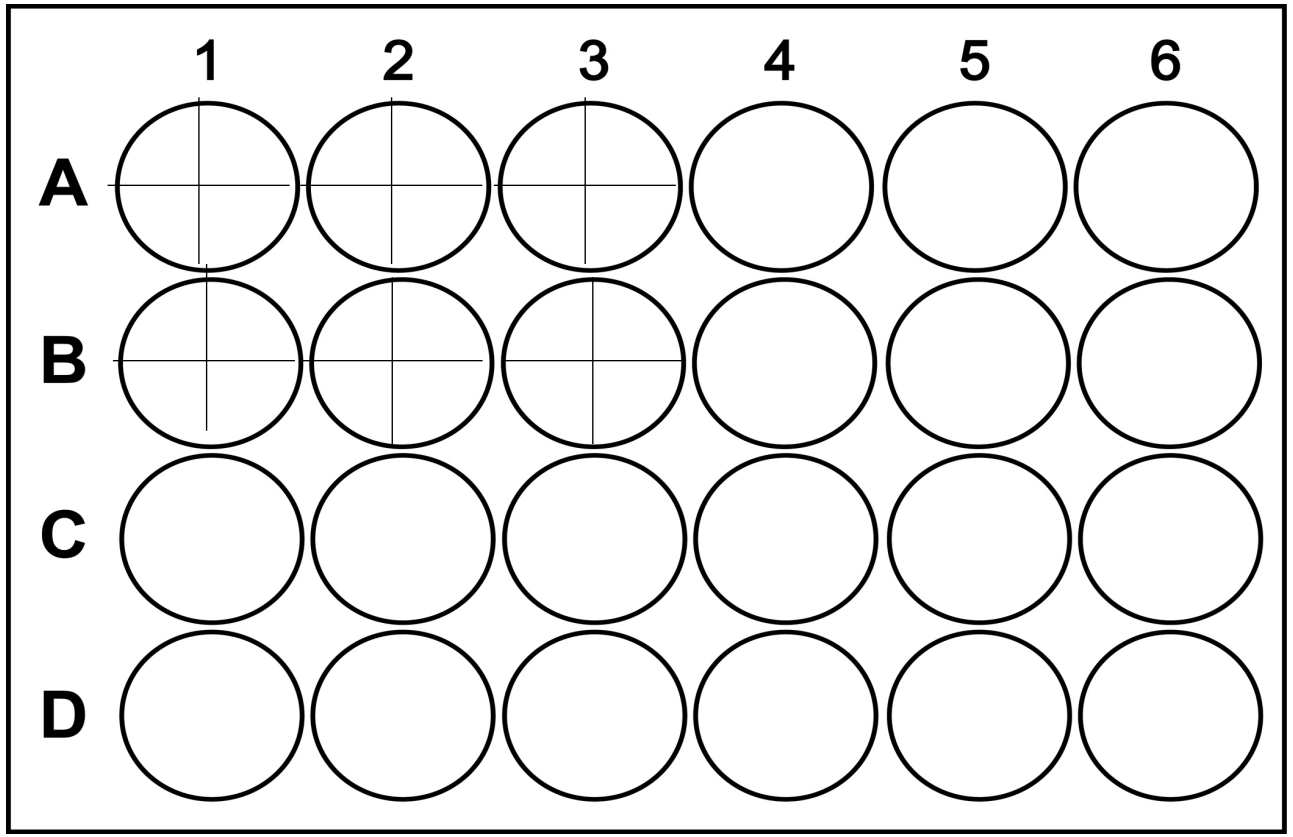
Variables

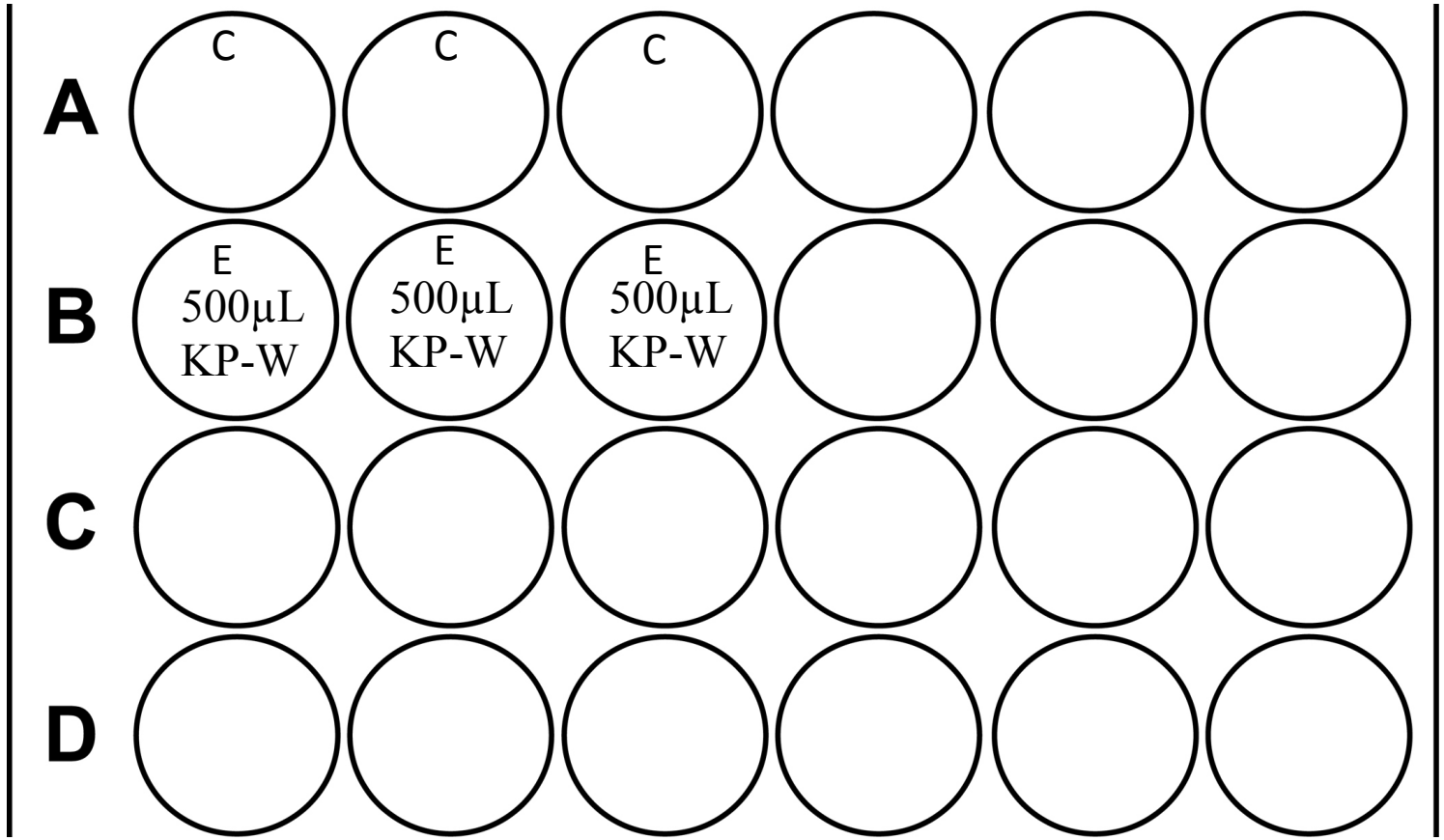
- Independent - Aqueous Solution (KP-W) treatment
- Dependent – Wound Healing percentage
- Constants – Cell line; Initial quantity of cells per well; Scratch width; Incubation period

http://www.cellsignet.com/media/plates/24.jpg

Methodology – Wound Healing Assay

Scratch is done with a micropipette tip and is 1mm in width.

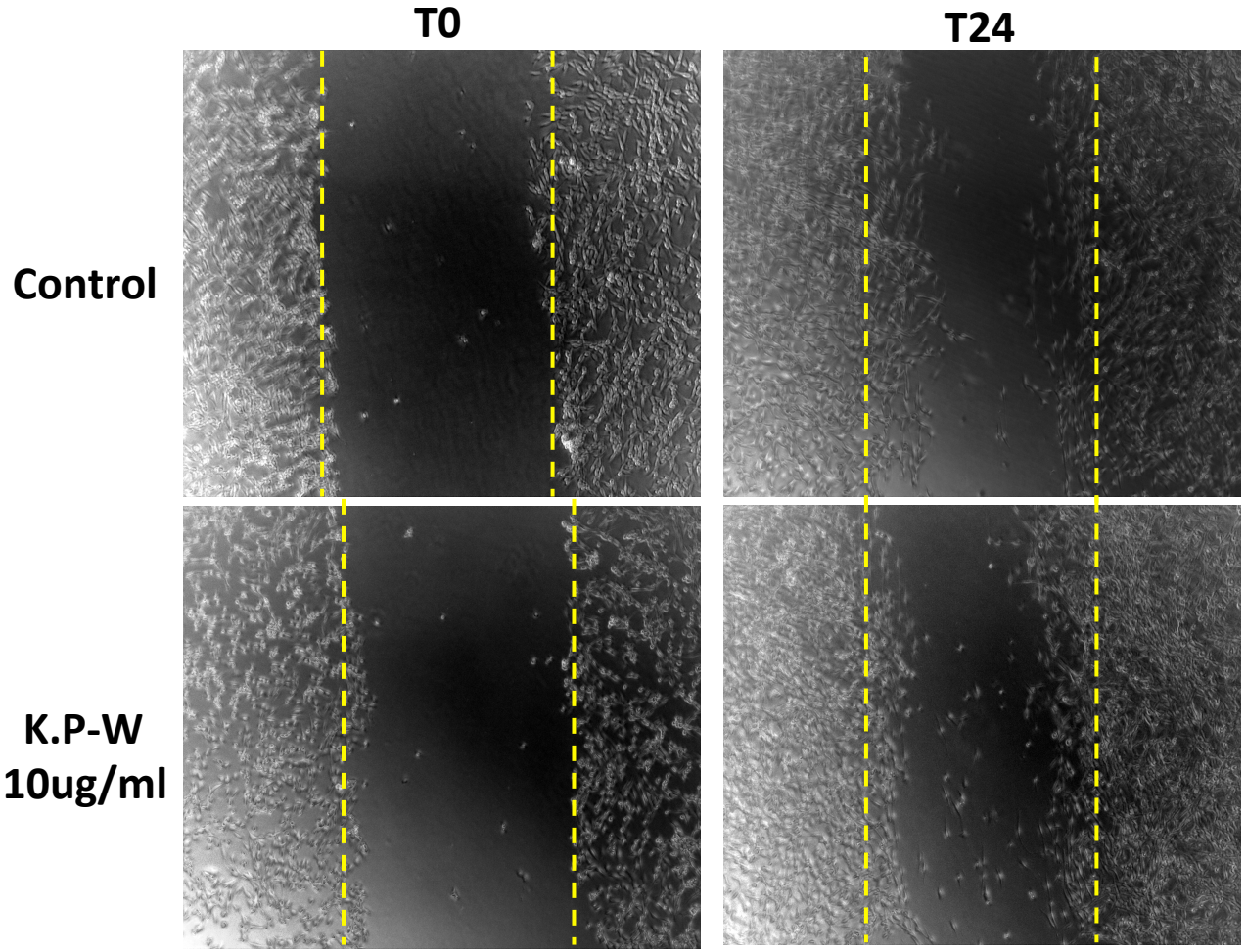


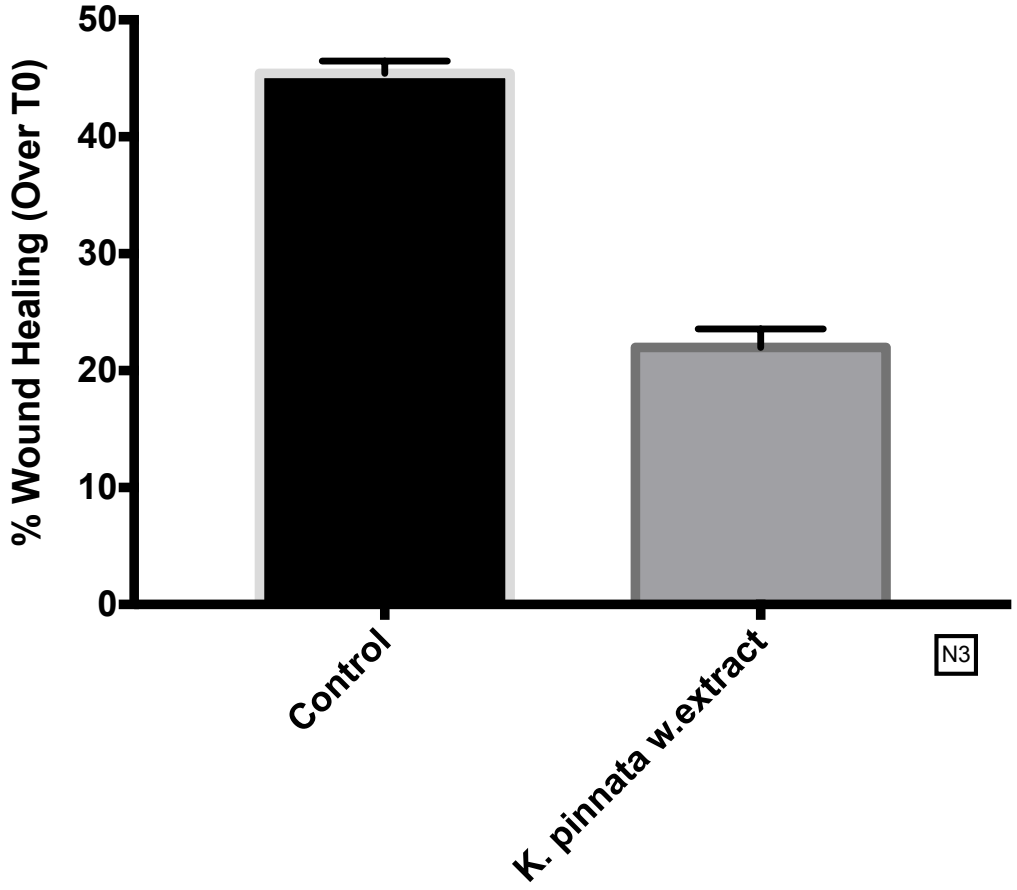


Methodology – Wound Healing Assay

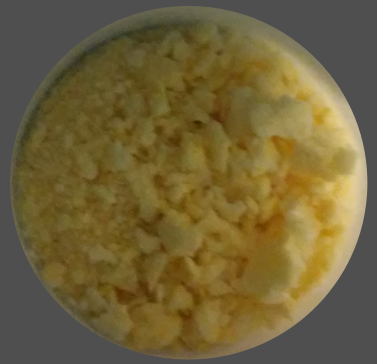
- Cells are treated with 500µL of KP-W and are incubated for 24 hrs.
- Cells are examined using Nikon Eclipse Ti microscope and images are taken using the Axiocam 105 Color. Analysis is done through Image J.

Wound Healing Assay Results & Analysis

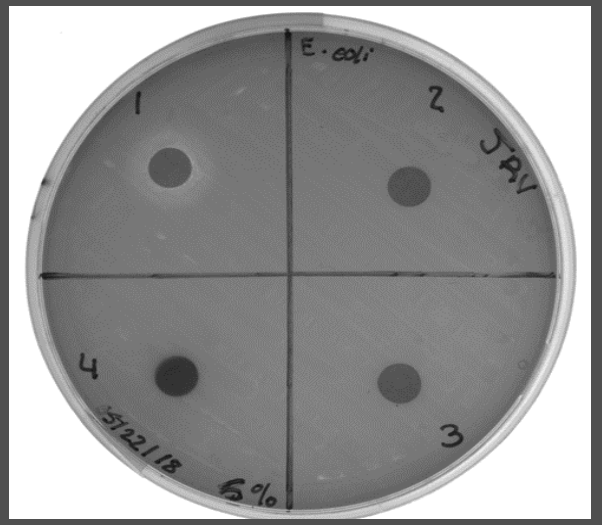




Summary

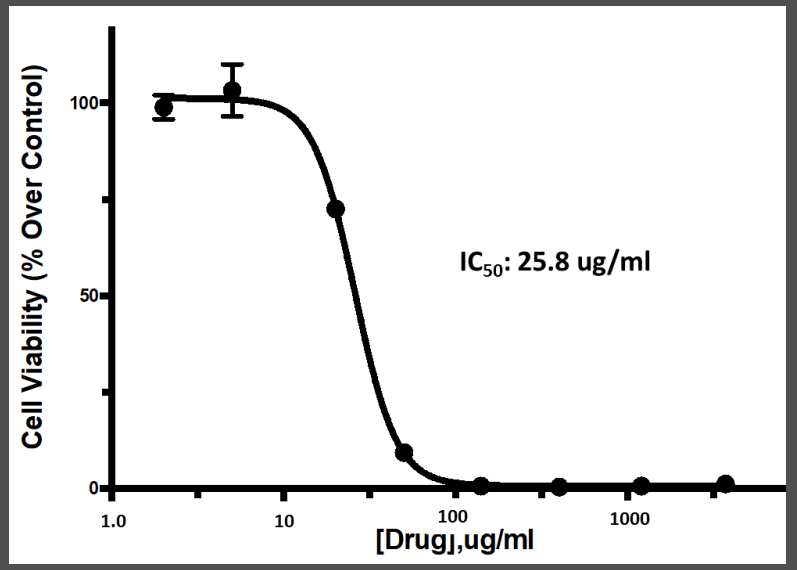


1. Extraction of Components

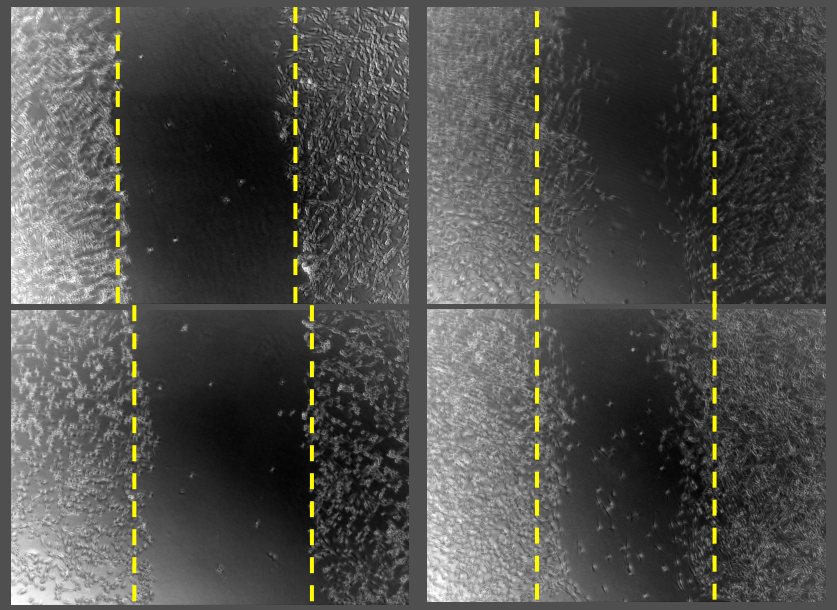


2. Antibiotic Susceptibility Test

3. Cytotoxicity Assay



4. Wound Healing Assay



Conclusion

- When in aqueous solution K. pinnata does not posses antibacterial qualities. However, it does maintain its highly cytotoxic components.
- K. pinnata has the ability to inhibit cell migration among astrocytomas when in aqueous solution but is only effective in small concentrations.

Future Research

Examining wound healing percentage of K.pinnata dissolved on a non-aqueous solution.

Comparing cytotoxicity and wound healing effects of K.pinnata on epithelial cells and astrocytomas.

Acknowledgements

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